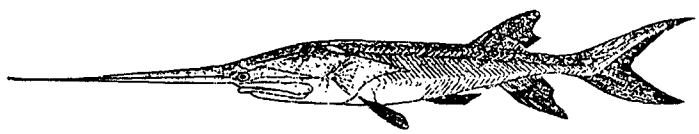
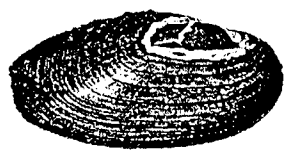
U.S. Fish and Wildlife Service
Region 3
Contaminants Program

Ohio River Islands National Wildlife Refuge: Examination of Contaminants using Mussels and Paddlefish as Indicators (3N25)





U.S. Fish and Wildlife Service
4469 - 48th Avenue Court
Rock Island, Illinois 61201
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Ohio River Islands National Wildlife Refuge: Examination of Contaminants using Mussels and Paddlefish as Indicators

(3N25)

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I. INTRODUCTION

The Ohio River Islands National Wildlife Refuge is located along almost 400 miles of the Ohio River from river mile 35 to 397 with headquarters stationed at Parkersburg, West Virginia. It is bordered by Ohio and West Virginia, Pennsylvania, Indiana, and Kentucky. Much of the lands bordering the refuge area are highly industrialized with many of these industries discharging to the Ohio River. Upstream of the headquarters, steel, coking, and coal mining are major industries. Downstream of the headquarters is the mouth of the Kanawha River which has a series of major chemical industries discharging into it. At river mile 282, the country's largest pulp and paper mill has been proposed for construction.

The Refuge provides important habitat for many species including paddlefish and mussels. This study proposes to examine paddlefish and mussels as bioindicators for pollution occurring in the Refuge. Paddlefish act as bioaccumulators of organochlorines because of their long life, their filter feeding habits, and their large fat reserves. Mussels are good bioindicators because they are localized and are filterfeeders. Pathways of contamination include the suspended and possibly dissolved fractions of the water column ingested through filter feeding, and sediments ingested through feeding on the bottom for both paddlefish and mussels. Another pathway includes contaminant accumulating invertebrates and plants ingested by paddlefish.

The paddlefish is an interjurisdictional fish and has been considered as a candidate (previously Category 2) for inclusion on the List of Endangered and Threatened Species. Paddlefish continues to be a species of management concern with potential for listing under the Endangered Species Act. Listing of the paddlefish would be probable if data proved that reproduction was significantly reduced by the effects of contaminants.

The Ohio River supports some of the richest freshwater mussel fauna in the United States including the federally endangered fanshell (*Cyprogenia stegaria)* and pink mucket pearly mussel (*Lampsilis abrupta)*. Mussel fauna are a major management interest of the Ohio River Islands National Wildlife Refuge.

The purpose of the study is to examine the extent of contamination at the Ohio River Islands National Wildlife Refuge with emphasis on PCB's and use paddlefish and mussels as bioindicators of this contamination. The study will specifically address the following:

1. The relative contamination of important resource areas on the Ohio River Islands NWR.

2. The impacts to trust species on the refuge from this contamination.

3. The implications for long term health and viability for those trust populations with emphasis on paddlefish and mussels. Parameters include reproductive success, health of the adult, and the pathways of exposure.

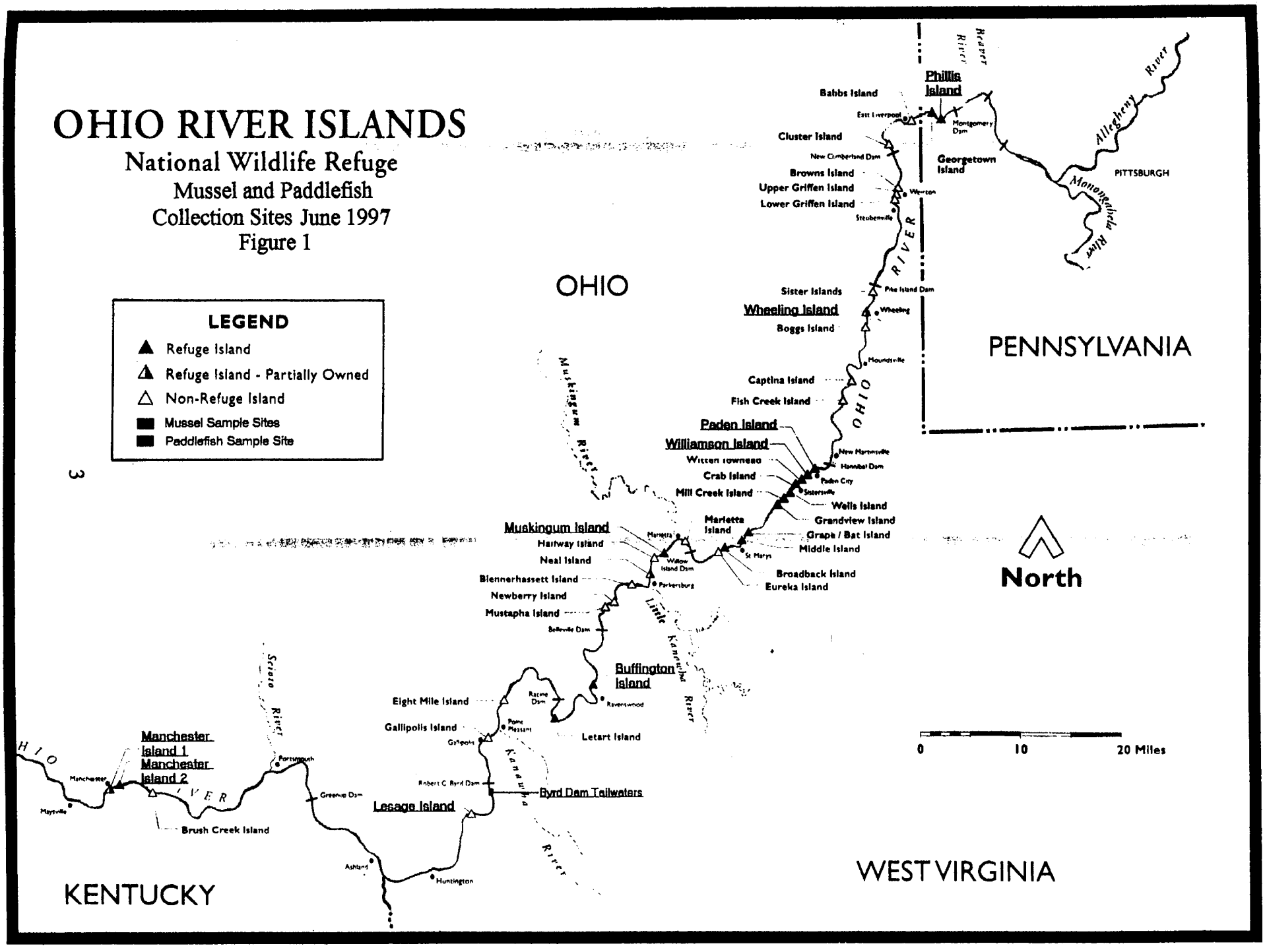
4. Added impacts from a large pulp and paper mill proposed at river mile 282 will be assessed at the request of West Virginia Field Office by examination of dioxins in paddlefish reproductive tissue.

5. The investigation will also validate biomarkers for paddlefish as indicators of fish health which have already been established for other fish species. The biomarkers are consistent with those used in the Biological Survey's Mississippi River Basin status and trends survey. These include hepatic and splenic macrophage aggregates, ethoxyresorufin-O-deethylase activity, plasma sex steroids using radioimmunoassay, and immunoquantitation of cytochrome P-450 1A1 using Western Blotting. Establishing these biomarkers for paddlefish will allow a broad assessment of paddlefish health to take place at reasonable costs.

The project was divided into two parts. Part A focused on the mussel resources of the Ohio River Islands National Wildlife Refuge and the extent of their exposure to contaminants as determined by soft tissue residue analyses. Part B focused on paddlefish. It is presented in the attached paper by Gundersen et al. (2000) preceded by our interpretive summary.

OHIO RIVER ISLANDS

National Wildlife Refuge
Mussel and Paddlefish
Collection Sites June 1997
Figure 1



PART A
Contaminant residues in Mussels of the Ohio River Islands National Wildlife Refuge

The Ohio River supports some of the richest freshwater mussel fauna in the United States, including the federally endangered fanshell (*Cyprogenia stegaria)* and pink mucket pearly mussel (*Lampsilis abrupta)*. The Ohio River Islands National Wildlife Refuge has developed a program to assess the mussel resources of the Ohio River and to restock historic beds with depleted species. The following results will provide the manager with information to aid in assessing potential environmental problems related to impacts to mussels. In general, it appears that harmful organochlorine pollutants are decreasing while potentially toxic metal concentrations are not decreasing as determined from residue analysis of sediments and mussel soft tissue compared with earlier results.

Sampling Locations

Mussel samples were taken from eight locations along islands of the Ohio River Islands National Wildlife Refuge. These include Phillis Island, Wheeling Island, Paden and Williamson Islands, Muskingum Island, Buffington Island, Lesage Island, and Manchester Islands. Mussels were collected from known mussel beds adjacent to those islands (Figure 1).

Sediments were collected at Phillis, Wheeling, Buffington and Lesage Islands. Collections were made on the main channel side of the islands, at the downstream end, in water less than 1 foot deep where silty, organic sediments were likely to accrete and in close proximity to mussels sampled.

Methods

Mussels were collected using a crowfoot brail bar towed downstream. In some locations, two boats, and two brail bars worked the mussel beds. Depending upon the density of the beds, the brail bars were dragged and redragged for a mile adjacent to a refuge island in areas thought to be most dense. Mussels were removed from the brail bars and retained in site water until the end of the field day. The mussels were then sorted for desired species and size. The desired specimens were opened with chemically cleaned tools, and the soft body parts and fluids were scraped into a chemically clean jar. Mussels of the same species were composited into the jars, with numbers composited depending on body mass.

Sediments were collected with a chemically cleaned stainless steel spoon in areas of accreting soft sediments near the brailling sites.

The jars were then frozen in a conventional freezer and shipped within a month to a Service

contracted laboratory. Metals and organochlorines were analyzed at the Service's Patuxent Labs and the dioxins were analyzed at Geochemical and Environmental Research Group at Texas A & M.

Results

Metals - Mussels

Soft tissue from mussels were analyzed from all sample locations for metals. Results for all samples exceeded the lowest detection limit (varying from 0.5 ppm for boron to 0.0006 ppm for cadmium) except for boron and beryllium (Table 1). Boron and beryllium concentrations, where detected, were low and remained close to the detection limits.

Maximum metal residue concentrations for mussel soft parts were highest for aluminum (1180 ppm), magnesium (1830 ppm), iron (8950 ppm), and manganese (10,800 ppm). Maximum concentrations of heavy metals were higher than those of many reference sites (Anderson 1977, Naimo et.al 1992) and were reflective of the concentrations of metals in the sediments.

Chromium values for the Wheeling mapleleaf (*Quadrula quadrula*) sample had a dry weight concentration of 33.3 ppm, about ten times higher than that of any other sample. Copper dry weight maximum concentrations of 52.7 ppm for mapleleaf at Phillis Island exceeded the values for copper at all other sites sampled by two to three times, and approach those found in river sites contaminated with lead mining tailings of 61 ppm (Schmidtt and Finger 1982). Zinc maximum concentrations of 633 ppm and 591 ppm for threeridge (*Amblema plicata*) mussels from Muskingum and Manchester Islands, respectively are more than double the values found for threeridge on the Upper Mississippi River (Naimo, et al. 1992).

Comparing our analyses of elephant ears (*Elliptio crassidens*) from Lesage Island (closest of the islands sampled to the Apple Grove site) with the 1990 analysis from one specimen of an elephant ear mussel from Apple Grove site, found most metals were comparable (Table 2). Aluminum, however, doubled in concentration in our analyses, from 520 ppm at Apple Grove to 1180 ppm at Lesage Island. Lead was detected at about 50% lower concentration, from 10.4 ppm at Apple Grove to a mean value of 5.99 ppm at Lesage Island. Vanadium increased by an order of magnitude from 0.63 ppm to a mean value of 5.50 ppm. Arsenic and selenium were both detected in this survey with mean values of 4.83 and 3.08 ppm respectively and were not either analyzed for or detected in the Apple Grove study.

Cadmium values for the Lesage Island elephant ear samples were much higher at a mean of 8.38 ppm (n=2) than for the other 19 samples with a mean of 2.23 ppm. Naimo, et al. (1992) found concentrations of cadmium in mussels (threeridge, *Amblema plicata*) of the Upper Mississippi River at a lightly contaminated site to range from 0.80 to 1.25 ppm. The upper value approximately equals the values for cadmium in threeridge at Paden and Williamson Islands (1.58 ppm) and Buffington Island (1.20 ppm) while the values for threeridge at

Muskingum (1.90 - 2.69 ppm) and Manchester Islands (2.50 ppm) are about twice as high.

Metals - Sediment

Concentrations of metals in sediments were highest for aluminum, iron, manganese, magnesium, and zinc. Concentrations of aluminum, arsenic, chromium, magnesium, nickel, and lead were greater in the sediments than in the residue whole bodies of the mussels. Copper sediments were greater than mussel soft bodies except for Phillis Island samples in which tissue and sediment concentrations were similar. Zinc concentrations in mussels were similar to concentrations in sediments. Cadmium, manganese, and selenium concentrations in sediment were less than that found in soft body mussel residue.

Heavy metal concentrations in Ohio River Islands NWR sediment are similar to waterways characterized as moderately polluted, based on classification of Illinois stream sediments (IEPA 1997). Sediment samples taken at Phyllis and Wheeling Islands contained concentrations of heavy metals exceeding values below which harmful effects are unlikely to be observed (MacDonald in press). These no effect concentrations were exceeded for arsenic, cadmium, chromium (Wheeling only), copper, mercury (Phillis only), nickel, lead, and zinc. No effect concentrations were also exceeded at Buffington and Lesage Islands for nickel and zinc.

Nickel analyzed from Phillis and Wheeling Island sediment samples was the only metal that exceeded the value (48.6 ppm) above which harmful effects are likely to be observed. Nickel concentrations ranged from 56 to 69 ppm at Phillis Island and 76.7 to 85.6 at Wheeling Island.

Organics

Total PCB's were not detected in any mussel tissue sample at any site at a 0.05 ppm (wet weight) detection limit. Samples taken in 1990 detected total PCB's in soft mussel tissue of elephant ear (0.12 ppm) and pimpleback (0.23 ppm) mussels downstream of Apple Grove, West Virginia, near our Lesage Island site.

Organochlorine compounds were not present above the detection limit (0.01 ppm wet weight) for all soft body mussel tissues samples. Two dioxins were detected in mussel soft tissue. OCDD was found at 130.71 ppt dry weight for a mapleleaf sample taken at Paden/Williamson; 229 ppt dry weight from a mapleleaf sample at Lesage Island; and 2,3,7,8-TCDF was detected in a mapleleaf sample from Paden/Williamson Islands at 12.14 ppt wet weight. Little is known about the OCDD or the 2,3,7,8-TCDF isomers. However, the 2,3,7,8-TCDD isomer is one of the most toxic compounds known (Eisler 1986). The 1990 analysis of mussel soft tissue from Apple Grove found 13 dioxins above the detection limit including 1,2,3,7,8-TCDD.

PCB's were found in sediments to exceed the detection limits varying from 78 to 111 ppb dry

weight at Phillis (237 to 553 ppb dry weight), Wheeling (512 to 689 ppb dry weight), and Lesage (174 to 180 ppb dry weight) Islands. These concentrations are considered to exceed the level below which harmful effects are unlikely (59.8 ppb). One sediment sample from Wheeling Island of 689 ppb dry weight exceeded the concentration above which harmful effects are likely (676 ppb) (MacDonald In press). PCB's were not detected in sediments taken at Buffington, the only other sediment site sampled.

Conclusions

Metal concentrations in soft tissue at the Lesage Island site were generally comparable with those taken 6 years previous at Apple River. Some metals were detected in higher concentrations in 1997 including aluminum, copper, nickel, selenium, and vanadium. Conversely, barium, lead and zinc were found at lower concentrations in elephant ear mussel tissue.

Metal concentrations in mussel soft tissue approach levels of concern, particularly zinc and copper. Zinc concentration in soft mussel tissue sampled at Manchester and Muskingum Islands had concentrations approaching 600 ppm dry weight. Copper in soft mussel tissue was found as high as 52 ppm dry weight at Phillis Island.

Cadmium concentrations in the large elephant ear mussels was notably greater than those for smaller sized specimens as Naimo et al. (1992) but we did not see a similar trend with zinc as they did.

Though the toxicity of these residue concentrations is not known, some heavy metals may be of concern for limiting mussel growth and reproduction. The interpretation is affected by ambient conditions which have a significant effect on the toxicity and bioavailabiliy of metals in a system.

It appears that harmful organochlorines may be diminishing based on uptake of mussel soft tissue. Compared to data six years previous, PCB's are concentrating at less than 50 ppb and other organochlorine pesticides were not detected. Only two dioxins were detected compared to 11 dioxin compounds detected in mussel soft tissue of two samples 6 years previous.

Phillis Island sediment was observed to exude an oily sheen when disturbed. PCB's were detected in the sediments of Phillis, Wheeling and Lesage Island sites. Though PCB's may have some adverse effect at all these sites, adverse effects from PCB's appear likely at Phillis Island site, based on MacDonald (In press).

Based on observation of oily substances exuding from the Phillis Island sediments, it is expected that significant concentrations of polyaromatic hydrocarbons maybe found, but were not analyzed in this study. These pollutants can be very damaging to the environment and may be limiting mussel productivity at Phillis Island.

Recommendations

Phyllis and Wheeling sites had the highest metal sediment concentrations of those sampled and are not recommended for reintroduction efforts at this time. Similarly, Phyllis and Wheeling Island sites exhibited some of the least dense mussel beds of those sampled. The refuge and Service field offices need to work with the states to continue to reduce metal discharges into the Ohio River system.

It is also recommended that Phillis Island be further examined for organic pollutants, like polyaromatic hydrocarbons. The appearance of an oily sheen during sediment sampling indicated that an oily substance, possibly toxic, is polluting the sediments. Efforts should also be made to link the pollutants with the discharger.

Organochlorines, however, do appear to be decreasing, as evidenced by lower residue concentrations in both mussels and paddlefish and in lower sediment concentrations compared to other studies. However, residual organochlorines may be continuing to have an adverse effect on paddlefish as evidenced by poor organ health (see Section B). The refuge should work with the field stations and states to insure that areas which may continue to contribute organochlorines from historic deposits are cleaned up and prevented from leaching into the system.

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Table 1. Concentrations of metals in freshwater mussels collected from the Ohio River Islands National Wildlife Refuge, June 1997. Results are reported in parts per million dry weight.

	Ph-1ML	Wh-1ML	Pw-1.3H	Pw-1.3R	Pw-1ML	Pw-2.3H	Pw-2ML
Al	822	1060	113	657	443	351	1140
As	3.90	3.60	5.80	4.80	3.30	4.70	3.60
B	<.900	<.700	<.800	<.700	<.800	<.800	<.700
Ba	112	306	325	234	403	211	297
Be	.060	.150	.082	.087	.120	.100	.120
Cd	1.14	4.12	1.45	1.58	3.21	1.43	2.26
Cr	4.40	33.3	3.40	4.70	5.10	8.60	5.00
Cu	52.7	15.8	21.8	17.6	15.5	16.1	15.8
Fe	1310	2850	1360	1460	3110	1330	3190
Hg	.092	.088	.100	.110	.180	.085	.150
Mg	1030	1150	1320	1060	1110	1170	1090
Mn	2020	4810	4760	2750	5010	2990	3570
Mo	1.00	1.50	.700	.900	.900	.800	.600
Ni	5.46	17.7	7.86	9.09	6.97	7.12	6.33
Pb	2.00	2.60	1.10	1.50	1.90	1.20	2.50
Se	2.60	2.00	3.70	3.00	3.30	3.60	2.70
Sr	64.1	119	135	116	134	95.4	103
V	2.5	5.00	3.10	2.90	4.20	2.30	4.50
Zn	147	244	98.7	185	195	85.9	155
% Ms	84.6	85.5	87.5	84.6	85.7	82.8	83.5

Table 1 cont. Concentrations of metals in freshwater mussels collected from the Ohio River Islands National Wildlife Refuge, June 1997. Results are reported in parts per million dry weight.

	Ls-1EE	Ls-1ML	Ls-2EE	Ma-1.3R	Ma-1ML	Ma-2.3R	Ma-2ML
Al	1180	1170	993	93	130	57.1	100
As	7.90	3.40	8.60	5.20	3.30	5.10	3.90
B	.800	1.00	<.600	<.600	.800	<.800	<.600
Ba	1360	358	1650	911	461	695	712
Be	.880	.110	1.00	.050	.040	.096	.060
Cd	8.44	1.90	8.32	3.21	2.10	2.50	3.20
Cr	11.0	4.00	11.0	8.80	6.60	5.50	19.8
Cu	12.0	16.0	13.0	7.40	15.0	5.44	16.0
Fe	7480	2800	8950	2780	2630	2050	4130
Hg	.210	.130	.230	.140	.140	.130	.170
Mg	1330	1160	1250	2170	1400	1830	1530
Mn	6060	4080	5870	10800	5020	7920	7040
Mo	1.40	.600	1.70	.910	.770	1.00	1.40
Ni	11.2	6.16	11.2	17.4	8.31	12.8	16.6
Pb	5.54	1.5	6.43	1.30	.940	.930	1.20
Se	4.00	3.00	4.40	2.70	3.20	2.30	3.20
Sr	211	124	215	513	231	393	294
V	5.70	4.50	5.30	6.60	3.00	5.00	4.40
Zn	168	176	172	591	205	434	244
% Ms	89.3	87.5	89.6	89.6	89.0	87.7	89.0

Table 1 cont. Concentrations of metals in freshwater mussels collected from the Ohio River Islands National Wildlife Refuge, June 1997. Results are reported in parts per million dry weight.

	Ls-1EE	Ls-1ML	Ls-2EE	Ma-1.3R	Ma-1ML	Ma-2.3R	Ma-2ML
Al	1180	1170	993	93	130	57.1	100
As	7.90	3.40	8.60	5.20	3.30	5.10	3.90
B	.800	1.00	<.600	<.600	.800	<.800	<.600
Ba	1360	358	1650	911	461	695	712
Be	.880	.110	1.00	.050	.040	.096	.060
Cd	8.44	1.90	8.32	3.21	2.10	2.50	3.20
Cr	11.0	4.00	11.0	8.80	6.60	5.50	19.8
Cu	12.0	16.0	13.0	7.40	15.0	5.44	16.0
Fe	7480	2800	8950	2780	2630	2050	4130
Hg	.210	.130	.230	.140	.140	.130	.170
Mg	1330	1160	1250	2170	1400	1830	1530
Mn	6060	4080	5870	10800	5020	7920	7040
Mo	1.40	.600	1.70	.910	.770	1.00	1.40
Ni	11.2	6.16	11.2	17.4	8.31	12.8	16.6
Pb	5.54	1.5	6.43	1.30	.940	.930	1.20
Se	4.00	3.00	4.40	2.70	3.20	2.30	3.20
Sr	211	124	215	513	231	393	294
V	5.70	4.50	5.30	6.60	3.00	5.00	4.40
Zn	168	176	172	591	205	434	244
% Ms	89.3	87.5	89.6	89.6	89.0	87.7	89.0

Table 2. Concentrations of metals in freshwater mussels collected from the Ohio River Islands National Wildlife Refuge, June, 1997. Results are reported in parts per million dry weight.

Analyte	Elephant ear mussel tissue [a]	Elephant ear mussel tissue sample 1 [b]	Elephant ear mussel tissue sample 2 [b]	Average of samples 1 [b] and 2 [b]
Al	520	1180	993	1087
As	nd	7.90	8.60	8.25
B	nd	0.800	<0.600	<0.700
Ba	2520	1360	1650	1505
Be	1.01	0.880	1.00	0.94
Cd	6.78	8.44	8.32	8.38
Cr	16.90	11.0	11.0	11.0
Cu	8.43	12.0	13.0	12.5
Fe	10700	7480	8950	8215
Hg	0.23	0.210	0.230	0.220
Mg	1410	1330	1250	1290
Mn	8150	6060	5870	5965
Mo	1.34	1.40	1.70	1.55
Ni	6.14	11.2	11.2	11.2
Pb	10.40	5.54	6.43	5.99
Se	--	4.00	4.40	4.20
Sr	336	211	215	213
V	0.63	5.70	5.30	5.50
Zn	258	168	172	170
%Ms	66.8	89.3	89.6	89.5

nd = none detected; lower limit of detection varied among compounds
-- = analysis not conducted

[a] Sample obtained downstream of Apple Grove, West Virginia, June 19-21, 1990 (Hudgins 1993)
[b] Samples obtained from Lesage Island, Ohio River Islands National Wildlife Refuge, June 1997.

Table 3. Concentrations of metals in sediments collected from the Ohio River Islands National Wildlife Refuge, June 1997. Results are reported in parts per million dry weight.

	Bf-1S	Bf-2S	Ls-1S	Ls-2S	Ph-1S	Ph-2S	Ph-3S	Ph-4S	Wh-1s	Wh-2s	TECs[1]	PECs[1]
Al	7870	10100	6530	7950	7880	10900	11900	10600	17400	13600		
As	7.00	7.50	6.30	7.20	9.20	11.0	11.0	12.0	13.0	13.0	9.79	33.0
B	.800	2.40	<.700	2.00	1.00	2.20	2.20	2.00	2.80	3.30		
Ba	94.8	105	97.7	119	91.0	118	140	124	193	176		
Be	.900	1.00	.870	.950	1.30	1.60	2.00	1.70	2.10	2.00		
Cd	.400	.400	.200	.200	.620	1.00	1.00	.910	1.40	1.00	0.99	4.98
Cr	20.0	23.0	16.0	20.0	29.0	30.0	39.0	39.0	45.0	36.0	43.4	111
Cu	25.0	27.0	22.0	20.0	34.5	46.5	52.4	52.9	60.2	53.5	31.6	149
Fe	25800	28800	22800	24500	35800	37300	53600	49100	45200	42500		
Hg	.078	.099	.071	.071	.071	.170	.360	.170	.150	.150	0.18	1.06
Mg	2230	2500	1970	2120	2230	2990	2400	2130	3490	3170		
Mn	1180	1140	882	902	938	1290	1800	1280	3250	2940		
Mo	.600	.500	<.500	<.500	1.00	1.80	3.40	2.70	1.00	.900		
Ni	38.0	40.0	32.0	33.0	56.0	59.0	69.0	60.0	85.6	76.7	22.7	48.6
Pb	26.0	28.0	26.0	23.0	49.0	62.0	59.0	69.0	67.0	60.0	35.8	128
Se	.350	.350	.270	.350	.430	.680	.720	.640	.940	.840		
Sr	27.3	31.9	19.4	27.7	33.6	37.0	31.2	33.5	50.4	37.1		
V	20.0	22.0	16.0	19.0	19.0	23.0	27.0	25.0	32.0	28.0		
Zn	140	149	127	131	226	286	336	305	381	347	121	459
% Ms	39.4	44.6	36.2	37.2	34.6	47.3	40.4	40.1	51.8	54.8		

[1] TECs = Concentration below which harmful effects are unlikely to be observed (MacDonald In Press).
PECs = Concentration above which harmful effects are likely to be observed (MacDonald In Press).

Table 4. Concentrations of total PCB's in sediments collected from the Ohio River Islands National Wildlife Refuge, June 1997.

	ppm dry wt.	Detect. Limit	ppm wet wt.	Detect. Limit	NOAA ER-L;-M [1]	TEC, PEC [2]
Bf-1S	<.0824	.0824	<.05	.05	.050, .400	.0598;.676
Bf-2S	<.0877	.0877	<.05	.05	.050, .400	.0598;.676
Ls-1S	.1805	.0778	.116	.05	.050, .400	.0598;.676
Ls-2S	.1745	.0793	.1099	.05	.050, .400	.0598;.676
Ph-1S	.2369	.0798	.1099	.05	.050, .400	.0598;.676
Ph-2S	.3591	.0911	.197	.05	.050, .400	.0598;.676
Ph-3S	.3734	.0854	.2185	.05	.050, .400	.0598;.676
Ph-4S	.5531	.0831	.333	.05	.050, .400	.0598;.676
Wh-1s	.6893	.1113	.3097	.05	.050, .400	.0598;.676
Wh-2S	.5122	.1109	.231	.05	.050, .400	.0598;.676

[1] ER-L = Effects Range Low
ER-M = Effects Range Median (Long and Morgan 1991)

[2] TEC = Concentration below which harmful effects are unlikely to occur
PEC = Concentration above which harmful effects are likely to occur
(MacDonald In Press)

Table 5. Dioxin concentrations in male paddlefish gonads (R3-R9) collected upstream of the Byrd Dam and in mussels (R-12 - R-18) from four locations* in the Ohio River Islands National Wildlife Refuge, June 1997, reported in parts per trillion, dry weight.

	HpCDD	HpCDF	HxCDF	PeCDF	PeCDF	TCDD	TCDF	OCDD
R3	14.69	21.62	31.19	24.09	23.97	32.67	91.42	79.04
R5	ND	21.40	40.43	15.81	21.52	7.73	86.68	46.02
R7	ND	17.86	35.03	23.82	28.20	30.12	102.98	49.04
R9	37.4	22.47	14.13	16.85	17.01	8.19	97.43	148.31
R-12	ND	ND	ND	ND	ND	ND	ND	ND
R-14	ND	ND	ND	ND	ND	ND	ND	ND
R-16	ND	ND	ND	ND	ND	ND	12.14	130.71
R-18	ND	ND	ND	ND	ND	ND	ND	14.76

R-12: Mapleleaf composite sample from Muskingum Island site.
R-14: Mapleleaf composite from Manchester Island site.
R-16: Mapleleaf composite from Paden and Williamson Islands site.
R-18: Mapleleaf composite from Lesage Island site.

ND: Below the detection limit

PART B
Contaminant residues in Paddlefish of the Ohio River Islands National Wildlife Refuge

SUMMARY

Fifty paddlefish (*Polyodon spathula)* were collected from 2 sites on the Ohio River and from 1 site on the Cumberland River to determine concentrations of PCB's and chlordane in the gonads. Gonad PCB and chlordane concentrations were significantly higher in Ohio River paddlefish than in Cumberland River paddlefish, making the Cumberland River a good reference site. There was a significant correlation between gonad organochlorine concentration (PCB's and chlordane) and gonad percent lipid content in Ohio River paddlefish. Five out of 10 Ohio River egg samples exceeded the Food and Drug Administration's (FDA) action limit for chlordane (0.30μ/g) with the other egg samples near this limit ($\geq$ 0.18). Percent hatch was not significantly different in eggs collected from the Cumberland (88 - 96%) and Ohio Rivers (90 - 95%). Plasma testosterone levels were significantly lower in males collected from the upper Ohio River site than males collected from the lower part of the river. Since PCB and chlordane concentrations in the testes of fish collected from the two Ohio River sites were not significantly different, other factors may be contributing to the low testosterone levels seen in fish collected from the upper part of the river. Liver, spleen, and kidney histology indicated immunosuppression, hepatic metabolic disorders and altered neuroendocrine function may be occurring in Ohio River paddlefish. Results from this study suggest that the long-term health of Ohio River paddlefish may be in jeopardy and further investigations on contaminant effects on immune function and hormone levels in paddlefish are warranted. Continued monitoring of edible tissues (eggs and fillets) is also recommended due to the high levels of chlordane found in the eggs.

It is noteworthy that only one female paddlefish was captured in the West Virginia site. Though many other dynamics may be involved, the low production of caviar in Eastern Europe has made paddlefish roe very valuable. In addition, organochlorines have bioaccumulated in the fatty tissue including the gonads. PCB's and chlordane may function as endocrine disrupters which ultimately could feminize the male fish, as indicated by reduced testosterone levels in fish high in organochlorines. However, sample fish spawned in the hatchery had a good success rate of hatch, though further developmental success is unknown. With the many pressure on paddlefish, it appears that an intensive monitoring program should

be conducted on the Ohio River Islands NWR for paddlefish to provide information on how best to manage this important species.

A report on this project, Biomarker response in polychlorinated biphenyl and chlordane contaminated paddlefish from the Ohio River Basin, USA by Deke Gundersen, Ruthellen Miller, Amy Mischler, Krista Elpers, Steve Mims, Jody Millar, and Vicki Blazer is attached. This report is scheduled for publication in the Society for Environmental Toxicology and Chemistry Journal in Volume 19, issue, 9, due out this August.

INTRODUCTION

The paddlefish is easily recognized as a unique fish with its smooth skin, long paddle-like snout and tail with elongated dorsal lobe. Only one other species is known from Polydontidae: the Chinese sturgeon *Psephurus gladius,* which inhabits the Yangtze-Kiang River in the Chinese lowlands (Becker 1983). As one of the largest freshwater fish, it can attain lengths of more than 1.8 m (5 feet, 11 inches), weigh more than 45.4 kg (100 pounds) and may exceed 30 years in age (Becker 1983). Paddlefish were historically abundant in most of the large rivers of the Mississippi River and Gulf Coast drainages. Since the turn of the century, significant declines in numbers have occurred. Dam construction and degradation of water quality have contributed to paddlefish stress. In the past few years, the paddlefish has become commercially valuable for its roe. Depression of sturgeon stocks in Eastern Europe has significantly increased the price of caviar and increased the pressure on paddlefish and sturgeon in North America (National Paddlefish and Sturgeon Steering Committee 1993). In 1992, the paddlefish was included in the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) to limit and monitor trade.

Paddlefish of the Ohio River have been the focus of conservation efforts by adjacent states and by the Fish and Wildlife Service, Ohio River Islands National Wildlife Refuge. Established in 1990, the Ohio River Islands NWR protects over 1100 acres of habitat including 19 islands located along 362 miles of river. Refuge areas serve as potential safe harbors for paddlefish, and as such are serving an increasingly important function.

However, water quality in the Ohio River may be limiting paddlefish productivity. The Ohio River has long been important as a transportation corridor, and in modern times has also acted as the catalyst for industrial development. Intensive development along its banks has resulted in abuses of its water and its ecosystems. One indicator of water quality stress is fish advisories. Fish consumption advisories based on PCB's and chlordane are in place for the entire length of the Ohio River. Kentucky is the only state that specifically identifies fish consumption advisories for paddlefish. Typically, fish containing contaminants at concentrations that yield fish advisories may also be impaired in some life function by that contaminant.

Fish and Wildlife Service data from 1993 in the vicinity of the Ohio River Islands NWR found

concentrations of PCB's in whole channel catfish at 3.5 ppm (FDA action limit is 2 ppm in fillets). Reproductive toxicity in fish has been shown to occur at whole-body PCB residues as low as 0.4 ppm (Eisler 1986). The Great Lakes International Joint Commission (1988) recommended 0.1 ppm total PCB's as a whole-body fish residue objective not to be exceeded to protect birds and mammals that consume fish.

Gundersen and Pearson (1992) found PCB concentrations in the roe of paddlefish at about 3 times the FDA action limit (15.97 to 42.99 ug/g lipid weight). These concentrations exceed those where reproductive effects in similar species have been found (Monod 1985). Monod found concentrations of 7.70 - 34.00 ug/g (lipid weight) in the eggs of Lake Geneva char correlated with a mortality rate of 29 - 100%.

Due to the rising concern for the protection of paddlefish stocks and the habitat required to sustain those stocks, we undertook an investigation to examine the extent of PCB and chlordane contamination at two areas on the Ohio River: the Ohio River Islands National Wildlife Refuge and Falls of the Ohio near Louisville. This study addressed the contamination of paddlefish on parts of the Ohio River and the implications for long term health and viability based on reproductive success, contaminant residues, and the establishment of biomarkers as indicators of relative health. The biomarkers were used in the National Biological Survey's Mississippi River Basin status and trends survey (Schmitt, et al. 1995). They include hepatic and splenic macrophage aggregates, ethoxyresorufin-O-deethylase activity, plasma sex steroids using radioimmunoassay, and immunoquantitation of cytochrome P-450 1A1 using Western Blotting. Establishing these biomarkers for paddlefish may permit a broad assessment of paddlefish health to take place at reasonable costs.

CONCLUSIONS

These results and the results of a previous study (Gunderson et al. 1998) indicate that PCB levels in the Ohio River may be declining. Ohio River paddlefish collected in 1998 were shown to have much higher egg and testes PCB concentrations (Gundersen and Pearson 1992). However, chlordane concentration in the gonads still exceeded FDA action limits of 0.3 ppm for edible tissue. DDT and its derivatives were not analyzed in this study.

Dioxins were found in all four paddlefish gonads analyzed. TCDD, one of the most toxic compounds known, was present in the four fish gonads sampled, ranging from 7.73 to 32.67 ppt.

Even though organochlorines may be reduced compared to previous levels, current concentrations may still be at levels which could present a health hazard as evidenced by poor organ health described in the attached paper.

Paddlefish from the Ohio River Islands NWR exhibited lower testosterone levels than those collected near Louisville or on the Cumberland River though PCB concentrations were

comparable among the Ohio River fish sampled. Paddlefish collected from the Byrd Dam were found to contain detectable concentrations of dioxin, including TCDD, though fish from other sample sites were not analyzed for dioxins. DDE may also be a causative factor, and was not examined in this study. It is not unlikely that organochlorines are contributing to suppression of testosterone levels in fish collected from the Byrd Dam.

RECOMMENDATIONS

1. The Refuge should work with the Service field station and the States to examine and clean up residual areas which may be contributing organochlorines to the system. If pollutants are adversely affecting the population, this factor needs to be considered for future management actions.

2. The FDA or other regulatory authority should be informed that some paddlefish gonads exceed the FDA action limit on edible tissue for chlordane.

3. The Refuge should work with the States and the Service fisheries offices to monitor and assess the paddlefish population of the Ohio River including sex ratio, age, size, reproductive success, etc. As fishing pressure continues to increase, basic information on the population and its viability will be needed to manage the fishery and to minimize overexploitation.

4. The contaminants of edible tissues (fillets, roe), immune functions, and hormone levels should be reassessed every five years to track fish health and the contaminant status of the Ohio River system.

LITERATURE CITED

See the References section for Gundersen et al. (2000) on page 24, attached.

Biomarker Response and Health of Polychlorinated Biphenyl and Chlordane Contaminated Paddlefish from the Ohio River Basin, USA

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ABSTRACT

Fifty paddlefish (*Polyodon spathula*) collected from 2 sites on the Ohio River and from 1 site on the Cumberland River, were examined to determine gonad polychlorinated biphenyl (PCB) and chlordane concentrations, amounts of plasma sex steroids (testosterone and estradiol), hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity, and the presence of immunoreactive cytochrome P450 1A (CYP1A) protein. Percent hatch and liver, spleen and kidney histology were also determined. Gonad PCB and chlordane concentrations were significantly higher in Ohio River paddlefish than in Cumberland River paddlefish. Gonad PCB and chlordane concentration and gonad percent lipid were correlated in Ohio River paddlefish. Five of 10 Ohio River egg samples exceeded the Food and Drug Administration's action limit for chlordane (0.30 μg/g). Polychlorinated biphenyl congener specific analysis detected predominantly the tetra-, penta- and hexachlorobiphenyls in paddlefish testes. Plasma testosterone levels were significantly lower in males collected from the upper Ohio River site than those collected from the lower part of the river. There was no measurable hepatic microsomal EROD activity in any of the 50 paddlefish collected from the 3 sites. Western blotting analysis confirmed that a rabbit anti-trout CYP1A1 IgG antibody did not recognize a CYP1A protein in paddlefish liver microsomes. Percent hatch was not significantly different in eggs collected from the Cumberland (88 to 96%) and Ohio Rivers (90 to 95%). Histological analysis of liver, spleen and kidney detected the presence of hepatic steatosis and hemosiderosis, splenic lymphoid cell depletion, and hyperplasia of interrenal and chromaffin tissues. Immunosuppression, hepatic metabolic disorders and altered neuroendocrine function may be occurring in Ohio River paddlefish. Results presented here suggest that organochlorine exposure may be jeopardizing the long-term health of Ohio River paddlefish and that additional investigation of contaminant effects on immune system function and hormone levels in paddlefish is warranted.

Keywords—Paddlefish Polychlorinated biphenyls Chlordane Reproduction Biomarkers

INTRODUCTION

The paddlefish because of its virtually scaleless body, long paddle-like rostrum and heterocercal tail is a unique fish. Only one other species is known from the family Polydontidae: the Chinese paddlefish *Psephurus gladius*, which inhabits the Yangtze-Kiang River in the Chinese lowlands [1]. The paddlefish is a large long-lived freshwater fish that can attain weights of more than 45 kg and individuals exceeding more than 15 years of age are not uncommon [2]. Typically most male paddlefish are not sexually mature until age 8 and most females do not mature until age 10 [2]. Paddlefish were historically abundant in most of the large rivers of the Mississippi River and Gulf Coast drainage. Since the turn of the century, significant declines in numbers have occurred. The decline of paddlefish populations is likely due to degradation of water quality and habitat destruction because of the use of the Ohio River as a transportation corridor, industrial development, and dam construction. Recently, the paddlefish has become a commercially valuable species due to the processing of its roe into caviar. The depression of sturgeon stocks in Eastern Europe has significantly increased the price of caviar and in turn increased the pressure on both paddlefish and sturgeon in North America [2]. In 1992, paddlefish were added to the Appendix II list of the United Nation's Convention on International Trade of Endangered Species of Wild Fauna and Flora [3]. This protection was designed to monitor the global import and export of products from paddlefish and curtail the illegal trafficking of caviar, which can be detrimental to wild populations of paddlefish. Further, the World Conservation Union/Species Survival Commission declared paddlefish as a vulnerable species because of decrease in population numbers and decline in habitat quality. Currently, the Mississippi Interstate Cooperative Resource Association (MICRA), which includes 22 states within the endemic range of the paddlefish, has coordinated several paddlefish studies among these states. Ohio River paddlefish have been the focus of conservation efforts by adjacent states and by the U.S. Fish and Wildlife Service, Ohio River Islands National Wildlife Refuge. Established in 1990, the Ohio River Island National Wildlife Refuge protects over 1100 acres of habitat including 19 islands located along 362 miles of river. Refuge areas serve as potential safe harbors for paddlefish and other threatened species, and as such are serving an increasingly important function.

However, Ohio River water quality may be limiting paddlefish productivity. One indicator of poor water quality is fish advisories. Fish consumption advisories based on polychlorinated biphenyls (PCBs) and chlordane are in place for the entire length of the Ohio River. Kentucky is the only state that specifically identifies fish consumption advisories for paddlefish. Fish containing organochlorines at concentrations that require the establishment of fish advisories, may be physiologically impaired by that contaminant. The U. S. Fish and Wildlife Service found whole body PCB concentrations in channel catfish at 3.5 ug/g in the vicinity of the Ohio River Islands National Wildlife Refuge [4]. Gundersen and Pearson [5] found PCB concentrations in the roe of paddlefish at about 2 times the FDA action limit (>4 ug/g). These concentrations exceed those where reproductive effects in similar species have been found [6]. Certain PCB congeners are also structurally similar to 2,3,7,8 – tetrachlorodibenzo-*p*-dioxin and have the ability to induce bioactivating enzyme systems [7], and some chemical components of these organochlorine mixtures are also suspected environmental endocrine disruptors [8].

Due to the rising concern for the protection of paddlefish populations and the habitat required to sustain these populations, we began an investigation to examine the extent of gonad PCB and chlordane contamination in paddlefish from two areas on the Ohio River: the Ohio River Islands National Wildlife Refuge and the Falls of the Ohio near Louisville, Kentucky and one site on the Cumberland River (a relatively less industrialized Ohio River tributary). This study was an attempt to determine if there was a relationship between gonad organochlorine contamination and certain biomarkers, which could be used to assess the long-term health and viability of Ohio River paddlefish populations. The biomarkers examined included observations of liver, kidney and spleen histology; quantitation of ethoxyresorufin-O-deethylase (EROD) activity; immunoquantitation of cytochrome P-450 1A; hatchability of fertilized eggs taken from contaminated fish; and measurements of plasma sex steroids. Establishing the validity of these biomarkers would permit a broad assessment of paddlefish health to be done at minimal costs.

MATERIALS AND METHODS

Paddlefish collections

Paddlefish, *Polyodon spathula*, were collected from three sites in the Ohio River Basin, USA, during the 1997 spawning season (April to October). Two of the sites were located on the Ohio River and the other site was located on the Cumberland River, an Ohio River tributary. A total of 50 paddlefish were collected from the 3 sites using large mesh gill nets (30 to 60 m in length, 4.8 m deep and 10 to 13 cm bar measure mesh). Nineteen paddlefish (8 males and 11 females) were collected from the Falls of the Ohio River, near Louisville, Kentucky, USA in the tail-waters of the McAlpine Dam (Ohio River mile 606.8). Eleven paddlefish (10 males and one female) were collected from the Ohio River Islands National Wildlife Refuge, near Hendersen, West Virginia, in the tail-waters of Robert Byrd Dam (Ohio River mile 279.2). Twenty paddlefish (8 males and 12 females) were collected from the Cumberland River, near Aaron, Kentucky, in the tail-waters of Wolf Creek Dam. Captured fish (alive) were weighed and measured for total length. Paddlefish not used for spawning experiments were killed by a blow to the head and the dentary bone was removed for age determinations as described by Gundersen and Pearson [5]. The gonads were removed and wrapped in aluminum foil and put on ice for transport to the laboratory where they were stored at -20°C. The liver, kidney and spleen were removed from each fish, and pieces fixed in 10% buffered formalin. The remaining liver portions from each fish were frozen in liquid nitrogen and stored on dry ice for transport to the laboratory where they were kept at -80°C for later EROD analysis. Blood was drawn using the vacutainer system (Franklin Lakes, NJ, USA) with lithium heparin as an anticoagulant. Blood samples (approximately 2 ml) were placed on dry ice for transport to the laboratory. At the laboratory, blood samples were centrifuged and the plasma was pipetted into a microcentrifuge tube for storage at -80°C.

Paddlefish spawning

Of the 50 fish collected, seven mature female paddlefish and 12 mature male paddlefish (from the 50 that were collected) were transported to the Aquaculture Research Center at Kentucky State University, Frankfort, Kentucky, USA, for spawning. Plasma was collected from each fish prior to transport.

Spawning of paddlefish and fertilization of eggs were done by injecting male and female paddlefish with a luteinizing hormone releasing hormone analog (LHRH-A; des-Gly[D-Ala6]-LHRH) at a dose of 0.05 mg/kg and 0.1 mg/kg respectively. Paddlefish ovulated 12 to 14 h after injection and males were actively spermiating 12 to 18 h after injection. The ovulated eggs from each female were stripped into a dry pan and the milt from males collected from the same site as the female was added and mixed. An aqueous suspension of Fuller's earth was added to the fertilized eggs in order to activate the spermatozoa and to prevent egg adhesion. The eggs were mixed and incubated at a water temperature of 18°C for 10 min. Several hundred eggs were then loaded into screened incubation units and maintained at 18 ± 0.3°C in an aerated water bath. The hatch success of eggs from each female was determined.

PCB and chlordane analysis

Extraction and cleanup procedures for paddlefish gonads were done based on the methods described by Gundersen et al. [9]. Subsamples of gonad homogenates (5 to 10g) were combined with sodium sulfate (approx. 50 g) and ground to a fine powder using a mortar and pestle. Dried tissues were Soxhlet-extracted (10 h) with 170 ml of 1:1 petroleum ether/hexane (v/v spectral grade, Sigma-Aldrich, St. Louis, MO, USA). Extracts were concentrated to less than 15 ml with a rotary evaporator and transferred to tared vials where the remaining solvent was evaporated to dryness using a warm water bath and stream of pure nitrogen (N_2). Lipid extracts were cleaned up using florisil columns (400 X 19 mm), and PCBs and chlordane were eluted with 6% ethyl ether/petroleum ether (v/v). Polychlorinated biphenlys and chlordane were separated using silica gel columns (10.5 x 300 mm); Polychlorinated biphenlys were eluted with hexane and chlordane was eluted with benzene.

The cleaned extracts were analyzed for total PCBs and chlordane by gas chromatography, using a Varian 3700 (Palo Alto, CA, USA) gas chromatograph equipped with a ^{63}Ni elecrtron-capture detector and a glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport (Supleco, Bellefonte, PA, USA). Isothermal gas chromatographic parameters were set as follows: Carrier gas, argon/methane (95%/5%), 60 ml/min; injector temperature, 240°C; detector temperature, 210°C; column temperature

200°C. Quantification of cleaned-up PCB fractions involved the use of an Aroclor 1254 external standard (Supelco, Bellefonte, PA, USA), that most resembled PCB mixtures in tissue extracts as determined by GC-MS analysis. A technical chlordane (Supelco, Bellefonte, PA, USA) external standard was used for quantifiying cleaned-up chlordane fractions. Quality assurance measures included the analysis of reagent blanks, duplicates, and spiked samples. Percent recovery in spiked samples was greater than 94% for total PCBs and 91% for chlordane, therefore sample extracts were not corrected for percent recovery. The Patuxent Analytical Control Facility (Laurel, MD, USA) analyzed two tissue homogenates for total PCBs (for inter-laboratory comparison) and other organochlorines. Total PCBs (reported as Aroclor 1254 by both laboratories) reported by the two laboratories differed by an average of less than 8%.

Some gonad extracts were analyzed for individual PCB congeners by gas chromatography-mass spectrometry (GC-MS) using a Varian Saturn II gas chromatograph coupled to a quadrapole ion trap mass spectrometer. The gas chromatograph was equipped with a Supleco SPB-5 capillary column (30 m X 0.25 mm i.d., 0.25 μm film) and had carrier gas (He) flow rate of 1 ml/min. The column temperature program started at 90°C for 5 min, raised at 20°C/min to 120°C, then raised to 300°C at 4°C/min and remained at the final temperature for 2.5 min. An Aroclor 1254 standard and individual PCB congeners (AccuStandard, New Haven, CT, USA) were used to identify PCB congeners in gonad extracts. GC-MS analysis indicated that the Aroclor 1254 standard was a close match to PCB mixtures in field samples.

Steroid hormone radioimmunoassays

Unextracted, unfractionated plasma from each fish was assayed for testosterone and estradiol with Diagnostic Systems Laboratories (Webster, TX, USA) Active Testosterone (DSL-4000) and Ultra-sensitive Estradiol (DSL-4800) Radioimmunoassay Kits. Subsequent procedures were performed according to the guidelines provided by the kit manufacturer. Results were reported as ng of testosterone per ml of plasma (ng/ml) and pg of estradiol per ml of plasma (pg/ml).

EROD assays and Western blotting

Liver sections were homogenized in 4 volumes (w/v) of ice-cold buffer (0.1 M tris-acetate, 0.1M KCl. 1.0 M EDTA, 20.0 μM BHT, and 0.1 mM PMSF; pH 7.4) and centrifuged at 10,000 g for 30 min followed by centrifugation of the supernatant at 100, 000 g for 90 min. Microsomal pellets were resuspended in 2 volumes (w/v) of buffer (0.1 M $K2HP0_4$, 1.0 mM EDTA, 20μM BHT, 0.1 mM PMSF, and 20% glycerol) and stored at -80°C until use in EROD assays and western blotting.

Ethoxyresorufin-O-deethylase was measured using the technique of Prough et al. [10] with 500 μg of microsomal protein. Enzyme activities were assayed on a Hitachi MPF-2A fluorometer using resorufin standards (Sigma, St. Louis, MO, USA). Cytochrome P450 1A (CYP1A) protein was analyzed by Western blotting using a polyclonal rabbit anti-trout CYP1A-IgG (gift of D.R. Buhler, Oregon State University, Corvallis, OR, USA). Microsomes were separated by sodium dodecyl sulfate-polyacrylamide gel elecrophoresis using 8% mini-gels (Jule Biotechnologies, New Haven, CT, USA) and the resolved proteins were transferred electrophoretically onto microporous polyvinylidene difluoride membranes (PVDF, Boehringer Mannheim GmbH, Germany) using a Hoefer transfer unit (model TE 70, San Francisco, CA, USA). Membranes were incubated with primary antibody (with 2% BSA) for 1 h followed by incubation in secondary antibody (rabbit Ig, horseradish peroxidase-linked antibody; Amersham, Arlington Heights, IL, USA) for 1 h. Cross-reactions were visualized with enhanced chemiluminescence (ECL) reagents (Amersham, Arlington Heights, IL, USA) detected by exposure to autoradiography film (Hyperfilm; Amersham, Arlington Heights, IL, USA). Protein concentrations were determined by the method of Lowry et al. [11]. Measuring EROD activity and cytochrome P450 1A protein in a female shovelnose sturgeon, a closely related species to the paddlefish, validated these methods. Hepatic microsomal EROD activity in this fish was 12 pmol/min/mg protein and there was cross-reactivity between a rabbit antitrout antibody and CYP1A protein in this species.

Histology

Pieces of liver, spleen, anterior and posterior kidney were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin (2 samples/tissue) and sectioned at 5 μm. Sections were stained with hematoxylin and eosin (H & E) and Perl's method for hemosiderin [12]. Sections were examined for tissue changes indicative of the effects of contaminant exposure or histologic biomarkers [13]. Tissue changes were classified as to type, and observations, of Perl's positive material (hemosiderin) within hepatocytes and macrophage aggregates, concentration of eosinophils within splenic white pulp, and hepatocyte vacuolization were rated on a scale of 0 to 4 (0 representing tissues that had no observable lesions or changes, 1 minimal lesions/changes, 2 mild, 3 moderate, and 4 severe). Mean ratings were compared among sites.

Statistics

Linear regression (least squares) analysis was used to determine correlation between paddlefish age and organochlorine (PCBs and chlordane) concentration, percent lipid and organochlorine concentration, and percent hatch and organochlorine concentration. Analysis of variance (comparison of all 3 sites) and a two-tailed, unpaired Student's t test (comparison between 2 sites) were used to determine site differences in organochlorine accumulation and plasma sex steroid concentration. Multiple linear regression analysis was used to determine which independent variables (mean gonad PCB and chlordane concentrations) were involved in the best fitting model for predicting plasma testosterone levels in Ohio River males. Significance level was $p \leq 0.05$ for all analyses.. Mean values were reported $\pm$ SD (standard deviation). All statistics were performed using Statgraphics® (Statistical Graphics Corp., Rockville, MD, USA) statistical software package for the IBM personal computer.

RESULTS

Paddlefish condition factors

Body condition factors were calculated for all 50 fish collected from the three sites. There was no significant difference in condition factors between Falls, Refuge and Cumberland fish (1.28 $\pm$ 0.20, 1.33 $\pm$ 0.10 and 1.47 $\pm$ 0.19 mm/g).

Gonad PCB and chlordane analysis

Gonad percent lipid, PCB and chlordane concentrations and age of paddlefish collected from the 3 sites are shown in Table 1. Gonad PCB and chlordane concentrations were higher in fish collected from the Ohio River (Refuge and Falls) when compared to those collected from the Cumberland River. Mean PCB and chlordane concentrations in the testes of Cumberland fish were significantly lower than those seen in the testes of Refuge and Falls fish (Table 2). Mean egg PCB and chlordane concentrations in Cumberland fish were also significantly lower than those seen in Falls fish. Comparison of immature ovaries could not be made since only one fish was collected from the Ohio River that had immature ovaries. Chlordane and PCB concentrations (wet weight basis) were highest in testes and immature ovaries, tissues that had a higher percent lipid content than eggs (Tables 1 and 2). However, when PCB and chlordane tissue concentrations were reported on a lipid adjusted basis, tissue concentrations (eggs, immature ovaries and testes) were similar. None of the 16 egg samples exceeded the Food and Drug Administration's action limit for PCBs in edible tissues (2 µg/g), but 5 egg samples did exceed the action limit for chlordane (0.30 µg/g). Egg samples that exceeded the Food and Drug Administration's action limit for chlordane came from paddlefish collected from the Ohio River (Table 1). There was no significant correlation between age and gonad organochlorine concentration when using wet weight tissue concentrations. However, there was a significant correlation between age and lipid adjusted gonad chlordane concentrations in Falls fish with eggs ($r^2 = 0.58$, correlation coefficient = -0.76, $p = 0.02$).

Three female and 7 male paddlefish from the Falls of the Ohio River, and 3 female and 5 male paddlefish from the Cumberland River were used for spawning experiments at the fish hatchery. The percent hatch of the fertilized eggs from each female is shown in Table 1. Percent hatch of fertilized eggs collected from both sites ranged from 90 to 95% for eggs from Falls fish and from 88 to 96% for eggs from

Cumberland River fish. Mean PCB levels (± SD) in the eggs of spawned Falls fish (0.58 ± 0.30 µg/g) were significantly higher than mean PCB levels in spawned Cumberland River eggs (<0.05 µg/g). Mean egg chlordane levels (± SD) were not significantly different between spawned eggs from Falls fish (0.26 ± 0.11 µg/g) and spawned eggs from Cumberland River fish (0.06 ± 0.03 µg/g). Mean gonad PCB and chlordane concentrations (± SD) were significantly higher in spawned males from the Falls (2.90 ± 1.56 and 1.01 ± 0.45 µg/g respectively) versus mean PCB and chlordane concentrations in spawned males collected from the Cumberland River (0.27 ± 0.21 and 0.23 ± 0.10 µg/g respectively). There were no significant differences in mean plasma testosterone and estradiol concentrations between spawned males or females collected from the Falls and the Cumberland River.

Thirty-five different PCB congeners were detected in the testes of paddlefish collected from the 3 sites (Table 3). Testes contained tetra-, penta- and hexa- chlorobiphenyls in the highest concentrations. Dominant PCB congeners (> 5% of total) found in samples from all 3 sites were 66, 118+123, 138 and 153 (Table 3). The pattern of chlorine substitution in the testes of all fish was quite similar to the pattern of substitution seen in an Aroclor 1254 standard.

Plasma sex steroids

Mean (± SD) plasma hormone levels (testosterone and estradiol) for male and female paddlefish collected from the 3 sites are shown in table 2. Mean plasma estradiol levels were not significantly different between females collected from the Falls and Cumberland sites. There was no significant difference in mean plasma estradiol levels between males collected from all 3 sites. The only significant difference in plasma testosterone levels was seen in Refuge males. Mean plasma testosterone levels in Refuge males were significantly lower than those seen in males or females from the Falls or the Cumberland River (Table 2). There was a significant negative correlation between mean plasma testosterone levels and gonad chlordane and PCB concentration in Refuge males (r^2 = 0.29 and 0.20, correlation coefficient = -0.54 and -0.45, p = 0.004 and 0.02 respectively). Of the variables looked at,

multiple regression analysis indicated that gonad chlordane concentration was the most important independent variable affecting plasma testosterone levels in male Refuge fish.

Cytochrome P450 analysis

There was no measurable hepatic microsomal EROD activity in the 50 paddlefish collected from the 3 sites. We were unable to detect CYP1A protein in paddlefish in a Western blotting analysis using rabbit antitrout CYP1A1 (LM_{4b}) IgG antibody.

Histology

Many observations were noted and rated during the histologic examination of the liver, spleen and anterior/posterior kidney. Only those for which differences among sites were noted will be presented.

Liver

Liver tissue of paddlefish from Cumberland River was composed of hepatocytes which were vacuolated (10 received a 4 rating, 3 a 3 and 6 a 2 rating) with eccentric nuclei (Figure 1a). Liver tissue from one fish had focal areas in which the cells were highly vacuolated and other areas with only +2 vacuolization. The hepatocytes contained varying amounts of Perl's positive material or hemosiderin (mean ± SD: 1.9 ± 0.9). Two of the fish contained large amounts of fat around vessels of the liver. Macrophage aggregates were also present in the liver of paddlefish. In fish from the Cumberland River there were many nonpigmented macrophages within these aggregates (Figure 1b). However, three pigments - hemosiderin, melanin and ceroid/lipofuscin could also be observed using the Perl's stain. Rating means for hemosiderin within macrophage aggregates was 1.9 ± 0.7.

Liver sections from Ohio River sites were more difficult to rate for vacuolization. The majority of the sections had an uneven distribution with foci of highly vacuolized cells whereas other areas were rated only 2 or 3. At the Refuge site 7 of 11 and at the Falls of Ohio River site 10 of 20 fish had focal areas of vacuolized cells. The less vacuolated areas had greater amounts of hemosiderin within hepatocytes while the vacuolated areas contained little or no hemosiderin (Figure 1b). Mean ratings for hepatocyte hemosiderin were 3.1 ± 0.6 for Refuge fish and 3.3 ± 0.6 for Falls fish. Macrophage aggregates were more numerous, larger and contained more pigmented cells at both these sites (Figure 1b) when compared to those collected from fish in the Cumberland River (Figure 1a). Mean ratings for macrophage aggregate hemosiderin were 3.2 ± 0.7 for Refuge fish and 3.1 ± 0.9 for Falls fish. Ten livers from the Falls site and two from the Refuge site contained large fat deposits around vessels.

Spleen

Splenic tissue in paddlefish was composed of both white and red pulp. The white pulp formed follicle-like masses around vessels and in most spleens from Cumberland River fish were composed primarily of lymphocytes, with some macrophages and eosinophils (Figure 2a). Three fish from this site had mild lymphocyte depletion of the white pulp. In contrast 15 of 20 Falls paddlefish and 10 of 11 Refuge fish had lymphocyte depletion ranging from mild to severe (Figure 2b). This lymphocyte depletion was often accompanied by an increase in the number of eosinophils within the white pulp (Figure 2b).

Anterior kidney

Anterior kidney of paddlefish contained both interrenal and chromaffin tissue, the functional equivalents of adrenal tissue in higher vertebrates. In paddlefish from the Cumberland River small clusters of interrenal cells were noted in two fish and a large cluster in one fish. The cytoplasm of these cells was eosinophilic, finely granular and nuclei contained prominent nucleoli (Figure 3a). Seven fish from the Falls

site had moderate to large clusters of interrenal cells, while four fish from the Refuge had large clusters and one had small clusters of interrenal cells. Interrenal cells from Ohio River fish were more vacuolated, ranging from slightly to highly vacuolated (Figure 3b). Nuclei were larger and stained more indistinctly.

Small clusters of chromaffin tissue (Figure 4a) were noted in five fish from the Cumberland River. In contrast there appeared to be a hyperplasia of chromaffin tissue in fish from the Ohio River. At the Refuge site one fish had a small amount of chromaffin tissue while 5 had moderate to large clusters of cells (Figure 4b). At the Falls site 3 had small clusters, while seven had the moderate to large clusters.

DISCUSSION

Gonad PCB and chlordane levels

The elevated gonad PCB and chlordane concentrations observed in Ohio River paddlefish compared to Cumberland River paddlefish were not unexpected since the Cumberland River is an Ohio River tributary that has little industry and urban development associated with it. These results are consistent with previous findings of low PCB levels in Cumberland River paddlefish [9]. Because Cumberland River fish had lower gonad PCB and chlordane concentrations this area was deemed an appropriate reference site.

The elevated PCB and chlordane concentrations seen in the testes and immature ovaries of Ohio River paddlefish probably were the result of the high lipid content of these tissues. While the lower organochlorine levels seen in paddlefish eggs can be attributed to the lower lipid content of these tissues. This was apparent when tissues were compared on a lipid-adjusted basis. Chlordane and PCB concentrations were similar when comparing the different reproductive tissues from each site on a lipid-adjusted basis. The female paddlefish reproductive cycle begins with a relatively small immature ovary that is associated with large fat bodies. This lipid rich tissue likely has a high organochlorine content that

becomes diluted as an egg mass develops and increases in size (1.5 to 4.5 kg). This large lipid reserve is used to produce an egg mass that may comprise 15 – 25% of the fish's body weight [2], resulting in lower organochlorine levels versus immature ovaries when reported on a wet weight basis. Gonad PCB and chlordane concentrations in our study are similar to levels seen in Ohio River paddlefish gonads from a previous study done in 1998 [9]. Ohio River paddlefish collected by Gundersen and Pearson in 1991 [5] had much higher egg (4.5 to 5.1 μg/g) and testis (5.6 to 23.0 μg/g) PCB concentrations than those presented here and those collected by Gundersen et al. [9]. These combined results indicate that PCB levels in the Ohio River may be declining.

The high gonad PCB and chlordane concentrations in Ohio River paddlefish were likely the result of several years of accumulation of these persistent compounds. Paddlefish examined in our study ranged from 6 to 17 years. Paddlefish probably bioaccumulate these organochlorines, with most residue uptake resulting from their feeding strategy. Paddlefish are filter feeders, trapping zooplankton in their large close-set gill rakers. As they ingest their food they also ingest detritus and suspended sediments, which can make up a large portion of the stomach content. We did not look at stomach contents of Ohio River paddlefish, but one study found that detritus and fine sediment made up over 50% of the stomach content of Missouri River paddlefish [14]. Since lipophilic compounds like PCBs and chlordane bind to particulate matter [15], it is possible that substantial levels of these contaminants are taken up by ingesting contaminated sediment. Another major source of contaminant uptake would be from ingestion of contaminated zooplankton. Little information exists on zooplankton surveys in the Ohio River. Previous research by Thorpe et al. [16], indicated that zooplankton richness and seasonal variation were not different among pools along the Ohio River including Cannelton pool, within our study area. In addition, since paddlefish migrate in open rivers and filter-feed on zooplankton indiscriminately [2], it is difficult to make comparisons on contaminant uptake between sites.

Female paddlefish may exhibit lower total organochlorine body burdens compared to males because these compounds depurate during spawning particularly when a female paddlefish can release between 6 to 10 lb of eggs. It is likely that a significant amount of their organochlorine body burden is

passed on to their offspring. Gundersen et al. [9] reported a negative correlation between gonad PCB concentration and age in female paddlefish collected from the Falls of the Ohio River. A negative correlation was seen in our study between gonad chlordane concentration and age in Falls females when using lipid adjusted values. Clearance of organochlorines via spawning was reported by Bengtsson [23] for minnows (*Phoxinus phoxinus*) and by Monod [6] for Lake Geneva charr (*salvelinus alpinus*), when lipid adjusted values were used. This depuration of organochlorines is probably insignificant in male paddlefish because there is a relatively small amount of milt released at spawning.

PCB congeners were analyzed in male paddlefish testes alone because at the Ohio River Islands National Wildlife Refuge, the primary emphasis of the study, only one female was collected from this site. Paddlefish testes contained primarily tetra-, penta- and hexachlorobiphenyls, which likely reflects the availability and persistence of these higher chlorinated congeners. Analysis of Lake Ontario smelt, *Osmerus mordax,* and Alewives, *Alosa pseudoharengus* (whole fish), showed they contained mainly tetra-, penta- and hexachlorobiphenyls [17]. These fish feed on a zooplankton diet similar to paddlefish.

Some of the PCB congeners (87, 99, 101, 118, 138 and 153) detected in Ohio River paddlefish testes made up a high percentage of the total detectable PCBs. These congeners are considered to be potentially toxic based on their structural similarities to 2,3,7,8 – tetrachlorodibenzo-*p*-dioxin and/or their ability to induce bioactivating enzyme systems [7, 18]. Some of these congeners induce cytochrome P450 – dependent mixed-function oxidases [19], however, no EROD activity was detected in any of the 50 paddlefish (discussed later). Additional research should examine the effects of these congeners on paddlefish physiology.

The high concentrations of chlordane seen in the eggs of Ohio River paddlefish may pose some concern for human consumption. Since paddlefish eggs are used to produce domestic caviar and 6 out of the 10 Ohio River egg samples exceeded the Food and Drug Administration's action limit of 0.30 μg/g for chlordane, public advisories may be warranted. These results are consistent with Gundersen et al. [9] who reported that 5 out of 6 Ohio River paddlefish eggs samples exceeded the Food and Drug Administration's

action limit for chlordane. These findings certainly suggest the need for inclusion of paddlefish roe in the various monitoring programs.

Paddlefish spawning

Previous studies in other species have shown that PCBs and chlordane have the ability to decrease egg hatchability [6,20-25]. Here, however, fertilization and percent hatch of paddlefish eggs from the Falls and Cumberland River were not significantly affected by the presence of PCBs and chlordane. Falls eggs had significantly higher PCB concentrations yet percent hatch was comparable to percent hatch in Cumberland River eggs (Table 2). These results are consistent with findings by Gundersen et al. [9], who showed that there were no affects on percent hatch in Ohio River paddlefish eggs contaminated with PCB (0.27 to 0.80 μg/g) and chlordane (0.24 to 0.56 μg/g) concentrations similar to those seen in our study. Polychlorinated biphenyl egg concentrations in Falls paddlefish are lower than PCB egg concentrations of 78 μg/g and 170 μg/g (wet weights) reported to adversely affect percent hatch in brook trout (*Salvelinus fomtinalis*) and minnow (*Phoxinus phoxinus*) eggs respectively [21, 23]. Nebeker et al. [26] found that female fathead minnows (*Pimephales promelas*) containing greater than 400 μg/g of aroclor 1254 had egg hatchability similar to controls. In addition, the effects of PCBs and chlordane on egg hatchability at the concentrations observed in our study are difficult to assess because limited information exists on the combined effects of these contaminants. The lack of a significant affect on hatchability may be due in part to this not being a sensitive toxicity endpoint in paddlefish.

Plasma sex steriods

Testosterone levels seen in Refuge males were lower than in other fish examined and there was a negative correlation between mean plasma testosterone levels and gonad chlordane and PCB concentration in Refuge males. However, it would be inaccurate to conclude that the gonad PCB and chlordane levels in

Refuge fish were the only factors responsible for the low plasma testosterone levels. Nevertheless, some components of these complex chemical mixtures are suspected environmental endocrine disruptors [8]. PCB induced hepatic hydroxylation of testosterone and inhibition of testicular steroidogenesis has been suggested as a major mechanism leading to depression of plasma androgen levels [27]. Brook trout, *Salvelinus fontinalis*, exposed to PCB (Aroclor 1254) contaminated water for 21 d had stimulated *in vitro* 11 β-hydroxylation of testosterone by testicular tissue [21]. Carp, *Cyprinus carpio*, and rainbow trout, *Oncorhynchus mikiss*, injected with 25 mg/Kg PCB (Aroclor 1254) showed a significant decrease in plasma androgen levels after 4 weeks [28]. Refuge males had significantly lower plasma testosterone levels than Falls males, yet fish from both sites had similar gonad PCB and chlordane levels. All Refuge and Falls males were determined to be sexually mature based on gross observations of the testes and the estimated age of the collected fish (Tables 1 and 2), but it is possible that males from the 2 sites were in different reproductive cycle stages. It is also possible that other contaminants may be involved. Typically contaminant levels are highest in the upper part of the Ohio River [29]. This is likely due to dilution by Ohio River tributaries. Polychlorinated biphenyl suspended sediment levels in the Ohio River were higher than those in all the major Ohio River tributaries from May 1988 to June 1990 [30]. Dichlorodiphenyltrichloroethane (DDT) metabolites were over 3.5 fold higher in the one Refuge fish analyzed for other organochlorines compared to a sample taken from a Falls fish. The persistent DDT metabolite p,p'- DDE has been identified as a potent environmental antiandrogen in mammals [31]. Since only one sample from each site (Falls and Refuge) was screened for other organochlorines we can not conclude that contaminants caused the lower testosterone levels seen in Refuge fish, particularly since several other factors may be involved.

Cytochrome P450

There was no measurable hepatic microsomal EROD activity in any of the fish collected from the Ohio and Cumberland Rivers. In addition, a Western blot analysis with rabbit ant-trout antibody failed to detect a CYP1A protein in paddlefish. These findings indicated that this biomarker may not be useful for the monitoring of exposure of this species to dioxins, organochlorines and polyaromatic hydrocarbons

(PAHs). Sex steroids, particularly estradiol, have been reported to influence monooxygenase activity in fish [32, 33]. Since most fish collected were in prespawning conditions, it is possible that this could have contributed to the lack of expression of EROD activity. However, it is difficult to conclude that plasma sex steroids were responsible for the lack of EROD activity in all 50 fish. Some males and females had considerably lower estradiol levels (54 to 184 pg/mL) than the other fish (250 to 402 pg/mL).

In an ongoing study in our laboratory (D.G. Gundersen, personal comminication), juvenile paddlefish (second year class) were injected with 5, 10, 20 and 40 mg/kg β - naphthoflavone, a known inducer of EROD in a number of species, and EROD activity was measured after 48 hr. None of the treated fish had measurable EROD activity. Similarly, in a study which examined TCDD contaminated Northern squawfish (*Ptychoceilus oregonensis*) Curtis et al. [34] reported little or no EROD activity in this species.

Histology

A number of microscopic differences were observed between paddlefish collected in the Cumberland River and those collected in the Ohio River. Unfortunately, there is very little information available on the normal histologic appearance of paddlefish organs and almost no information available on the response of various organs to toxicant exposure. The microscopic anatomy of paddlefish differed from most teleosts in a number of ways. First, the spleen had well defined white and red pulp as described for sturgeons [35] but no macrophage aggregates as are usually found in the splenic tissue of teleosts. Rather, foci of macrophages, lymphocytes and sometimes eosinophils were found in the liver in association with clusters of pigment-containing macrophages. The three pigments - hemosiderin, ceroid/lipofuscin and melanin - seen in other fish macrophage aggregates were observed within these pigmented macrophages. In teleosts the interrenal and chromaffin tissues are concentrated mainly in the anterior portion of the kidney while in paddlefish it was dispersed throughout the length of the kidney. These findings were in agreement with those of Rahn [36] who reported interrenal tissue throughout the kidney of paddlefish.

Many of the histologic changes we observed in paddlefish from the Ohio River have been previously documented as responses to contaminants in other fishes. The liver is important in normal digestion and storage of lipid and glycogen, xenobiotic metabolism and excretion, and production of yolk protein. For these reasons histopathologic changes which serve as biomarkers in the liver have received much attention. In higher teleosts two types of hepatic changes, hepatocellular steatosis or fatty change and hepatocellular hemosiderosis [37-39] are found in fish collected from contaminated sites. These intracytoplasmic storage disorders suggest altered metabolism in these fish. Hepatocytes of Cumberland River fish were highly vacuolated, probably as a consequence of the liver being very important in lipid and glycogen storage. Livers of Ohio River paddlefish had less vacuolization and greater amounts of hemosiderin. There were focal areas of more vacuolated cells, which resembled the clear or vacuolated altered cell foci in other fishes. As in altered cell foci from English sole [37], the foci in paddlefish showed a marked decrease in hemosiderin (Figure 3b). Steatosis/hemosiderosis of sole have shown consistent, statistically significant associations with polycyclic aromatic hydrocarbons in bottom sediments [40]. The histopathologic changes seen in Ohio River paddlefish liver may be associated with the higher levels of organochlorines seen in these fish versus Cumberland River paddlefish.

Increases in macrophage aggregate number/density have been noted in numerous fish species collected at contaminated sites versus those collected at reference sites [41]. Splenic and hepatic macrophage aggregates of centrarchids were more prominent at sites in Tennessee containing high levels of PCBs in sediment and biota when compared to a reference site [42]. Because this lymphoid depletion was noted only at the contaminated sites, it was believed to be related to contaminant exposure [42]. Both findings have previously been reported to relate to immunosuppression or potential defects in disease resistance [43]. However, further studies on infectous disease prevalence, functional assessment of lymphocytes, macrophages and the specific immune response would be necessary to determine if paddlefish from the Ohio River are indeed immunosupressed.

Although the stress response, specifically circulating cortisol, has been examined in contaminant related studies, few investigators have examined interrenal or chromaffin tissue histologically. Interrenal cells are homologous to the adrenal cortex of higher vertebrates and are responsible for the synthesis of corticosteroids, primarily cortisol. Chromaffin tissue is equivalent to adrenal medulla and contains the catecholamines epinephrine (adrenaline), norepinephrine (noradrenalin) and dopamine [44]. Bromage and Fuchs [45] found an increase in cell size, nuclear diameter and nucleolar size and number in goldfish exposed to sodium lauryl sulphate. Degenerative changes were found after exposure to higher concentrations (10 and 15 mg/l) and to two levels (2 and 3 mg/l) of zinc sulfate. They also report an apparent reduction in the thickness of the layers of interrenal cells around veins and the number of areas of interrenal cells [45]. Ram and Singh [46] found initial hypertrophy of cells followed by degeneration in interrenal and chromaffin cells of *Channa punctatus* exposed to ammonium sulfate. Donaldson et al. [47] reported an increased interrenal nuclear diameter in salmonids exposed to a variety of contaminants. In paddlefish, we observed some hypertrophy of interrenal cells. More importantly, however, there was an apparent hyperplasia or increased cell number in interrenal tissue and to a greater extent in chromaffin tissue. There appeared to be more foci of these cells and the foci were larger in Ohio River paddlefish. This is interesting in that PCBs have been shown to lead to dopamine depletion and neurotoxicity in other animals [48]. We were concerned that this response may have occurred in response to transport of selected fish for spawning. However, there was no correlation between increased interrenal or chromaffin tissue and transport. In addition, Barton et al. [49] found that paddlefish exhibit a much lower physiological stress response to physical disturbances than those documented for many teleost fishes. Effects of contaminants on endocrine systems in fish have primarily centered on reproductive hormone disorders. however perhaps more research should be directed toward examining the ability of these chemicals to exert neuroendocrine effects.

In summary, gonad PCB and chlordane levels were relatively high in Ohio River paddlefish, particularly in the testes of male fish which was correlated with the higher lipid content of these tissues. Egg chlordane levels were higher than the Food and Drug Administration's action limit for chlordane in 5 out of 10 Ohio River egg samples. Polychlorinated biphenyl congener specific analysis of Ohio River

paddlefish testes showed that these tissues largely consisted of the tetra-, penta-, and hexachlorobiphenyls. Some of these congeners have been shown to induce hepatic microsomal EROD activity in other fish species but we did not detect EROD activity in any of the 50 fish collected. This finding was substantiated by the lack of cross-reactivity between a rabbit antitrout CYP1A1 IgG antibody and a CYP1A protein in paddlefish microsomes. CYP1A does not appear to be a useful biomarker for contaminate exposure in this species. Percent hatch of fertilized paddlefish eggs from the Ohio River was good (90 to 95%), indicating that the PCB (0.38 to 0.93 μg/g) and chlordane (0.18 to 0.38 μg/g) levels in these eggs did not significantly affect this reproductive parameter. Plasma testosterone levels were significantly lower in males collected from the Refuge site compared to males collected from the lower part of the river. These significantly lower testosterone levels may be due to the presence of other contaminants in the upper part of the Ohio River. Histologic examination of paddlefish tissues (liver, spleen and kidney) suggest that Ohio River paddlefish could experience chronic health problems due to immunosuppression, and altered hepatic and neuroendocrine (adrenal) function. Continued investigations on Ohio River paddlefish health are warranted, particularly since the status of Ohio River paddlefish populations is questionable. Continued monitoring of edible tissues (roe and fillets) for persistent contaminants to establish health-based consumption advisories is also recommended since paddlefish are a long-lived species that have the potential to accumulate significant levels of organochlorine contaminants.

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Table 1. Age, gonad percent lipid content (% lipid), gonad PCB and chlordane concentration, plasma testosterone (T, ng/ml) and estradiol (E_2, pg/ml) concentration of paddlefish collected from the Falls (F), Refuge (R) and Cumberland (C) sites.

			Tissue PCB and chlordane concentrations						
			μg/g Lipid		μg/g Wet tissue				
Sample # - tissue	Age	% Lipid	PCBs	Chlordane	PCBs	Chlordane	T	E_2	%H[d]
F4, eggs	13	14.0	5.70	3.00	0.80	0.42[a]	20.1	394.4	-
F5, eggs	14	11.1	6.82	2.69	0.76	0.30	19.8	398.6	-
F6, eggs	15	24.0	6.39	2.46	1.53	0.59[a]	20.8	339.8	-
F7, eggs	10	12.8	7.16	3.50	0.92	0.45[a]	22.9	387.9	-
F8, eggs	13	11.5	5.15	3.14	0.59	0.36[a]	19.1	390.1	-
F11, eggs	10	5.8	5.51	4.65	0.32	0.27	20.8	399.2	-
F13, eggs	16	7.8	4.84	2.29	0.38	0.18	20.4	377.9	92
F15, eggs	16	7.8	5.35	2.68	0.42	0.21	23.7	384.4	95
F19, eggs	9	12.1	8.07	3.30	0.93	0.38[a]	18.4	375.1	90

F2, testes	12	20.0	18.66	7.69	3.74	1.54	17.9	391.1	-
F3, testes	17	70.8	12.25	3.01	8.68	2.13	10.2	402.5	-
F9, testes[b]	12	70.9	4.08	1.38	2.90	0.98	10.0	393.8	-
F10, testes[b]	11	38.4	9.91	2.24	3.81	0.86	20.2	381.1	-
F12, testes[b]	11	47.4	7.66	3.08	3.63	1.46	18.7	385.2	-
F16, testes[b]	16	11.2	12.6	5.29	1.43	0.60	22.6	357.4	-
F17, testes[b]	12	39.0	14.01	4.48	5.47	1.75	21.1	365.7	-
F18, testes[b]	13	58.5	1.61	0.84	0.94	0.49	18.3	358.8	-
F20, testes[b]	14	43.2	4.93	2.20	2.13	0.95	10.1	354.9	-
F14, IMO[c]	11	5.8	5.51	4.65	3.57	1.24	23.3	351.4	-
R1, testes	11	70.9	2.85	1.55	2.02	1.10	3.9	398.7	-
R2, testes	16	44.9	9.18	4.01	4.12	1.80	8.4	183.9	-
R3, testes	11	42.9	8.58	3.29	3.68	1.41	15.6	251.1	-
R4, testes	10	49.2	8.23	2.80	4.05	1.38	14.1	383.7	-
R5, testes	9	73.0	3.31	1.75	2.42	1.28	14.8	330.5	-

R6, testes	8	72.0	1.74	1.10	1.24	0.78	8.4	384.7	-
R7, testes	17	44.0	7.82	3.23	3.44	1.42	15.9	53.6	-
R8, testes	17	28.3	9.01	4.20	2.55	1.19	14.6	373.9	-
R9, testes	9	63.8	5.50	2.15	3.51	1.37	2.8	389.4	-
R10, testes	10	68.6	12.10	3.911	8.30	2.92	3.1	365.0	-
R11, eggs	10	12.6	2.94	1.98	0.37	0.25	5.6	361.4	-
C8, eggs	9	7.4	4.30	1.61	0.32	0.12	16.8	376.3	-
C10, eggs	15	21.5	<0.05	0.33	<0.05	0.07	23.9	372.4	88
C13, eggs	12	10.5	0.89	0.43	0.07	0.03	24.9	357.3	-
C15, eggs	12	18.3	<0.05	0.27	<0.05	0.05	23.9	363.5	-
C17, eggs	9	12.0	<0.05	0.50	<0.05	0.06	16.4	377.9	-
C18, eggs	11	11.6	<0.05	<0.05	<0.05	<0.05	17.1	379.5	-
C19, eggs	11	11.3	<0.05	0.70	<0.05	0.08	16.9	383.4	96
C20, eggs	8	10.4	<0.05	<0.05	<0.05	<0.05	17.3	385.9	93
C3, IMO	13	71.5	0.49	0.08	0.35	0.06	14.6	295.3	-

C4, IMO	7	71.6	0.31	0.22	0 22	0.16	15.4	368.2	-
C5, IMO	7	74.3	0.08	0.40	0 06	0.30	14.6	366.5	-
C7, IMO	9	76.9	0.17	0.31	0.13	0.24	16.9	24.5	-
C1, testes	6	81.0	0.63	0.12	0.51	0.10	17.8	161.3	-
C2, testes	9	64.7	0.40	0.08	0.26	0.05	19.8	60.9	-
C6, testes	11	52.7	0.61	0.21	0.32	0.11	20.9	364.8	-
C9, testes[b]	7	70.0	0.58	0.19	0.41	0.13	15.8	372.9	-
C11, testes[b]	7	66.3	0.48	0.57	0.32	0.38	21.6	370.9	-
C12, testes[b]	8	72.0	<0.05	0.29	<0.05	0.21	24.1	356.3	-
C14, testes[b]	6	10.4	5.01	2.80	0.52	0.29	12.0	371.2	-
C16, testes[b]	8	61.9	<0.05	0.24	<0.05	0.15	20.0	368.5	-

[a]Exceeds Food and Drug Administration's action limit of 0.30 µg/g, for edible tissues.

[b]The milt of these males was combined and used to fertilize eggs of females from the same site.

[c]IMO = immature ovary

[d]%H = percent egg hatchability from corresponding female.

[b]Mean plasma testosterone levels in Refuge fish are significantly different from plasma testosterone levels in Cumberland and Falls fish.

[c]Mean gonad PCB and chlordane concentrations in Cumberland fish are significantly different from corresponding tissue concentrations in Falls and Refuge fish.

Table 2. Mean (± SD) values from tissue analyses, and mean age, plasma testosterone (T) and estradiol (E_2) concentrations of paddlefish collected from the Falls (F), Refuge (R) and Cumberland (C) sites.

				Mean PCB and Chlordane Concentrations					
				(μg/g lipid)		(μg/g wet tissue)			
Site	Tissue	Age	% Lipid	PCBs	Chlordane	PCBs	Chlordane	T (ng/ml)	E_2 (pg/ml)
F	Eggs	13 ± 3	11.9 ± 5.3	6.08 ± 0.99	3.07 ± 0.70	0.74 ± 0.38	0.35 ± 0.13	20.9 ± 1.8	383.0 ± 18.2
	Testes	13 ± 2	44.4 ± 20.5	9.54 ± 5.46	3.19 ± 1.80	3.64 ± 2.34	1.19 ± 0.55	16.9 ± 4.9	372.5 ± 21.4
R	Testes	12 ± 3	55.8 ± 15.8	6.83 ± 3.33	2.80 ± 1.11	3.53 ± 1.92	1.46 ± 0.57	9.7 ± 5.4	306.9 ± 106.1
C	Eggs	11 ± 2	12.9 ± 4.6	0.92 ± 1.38[c]	0.53 ± 0.47[c]	0.09 ± 0.09[c]	0.06 ± 0.03[c]	18.2 ± 3.7[b]	374.5 ± 9.8
	IMO[a]	9 ± 3	73.6 ± 2.6	0.26 ± 0.18	0.25 ± 0.14	0.19 ± 0.12	0.19 ± 0.10	15.4 ± 0.9	263.6 ± 141.2
	Testes	8 ± 2	59.9 ± 21.6	0.98 ± 1.64[c]	0.56 ± 0.91[c]	0.30 ± 0.18[c]	0.18 ± 0.11[c]	19.0 ± 3.8	311.5 ± 116.5

[a]IMO = Immature ovaries

[b]Mean plasma testosterone levels in Refuge fish are significantly different from plasma testosterone levels in Cumberland and Falls fish.

[c]Mean gonad PCB and chlordane concentrations in Cumberland fish are significantly different from corresponding tissue concentrations in Falls and Refuge fish.

Table 3. PCB congener analysis (percent of total detectable PCBs) of paddlefish testes collected from the Ohio River Islands National Wildlife Refuge (R), the Cumberland River (C) and the Falls of the Ohio River (F).

IUPAC no.	Structure	R3	R4	C2	F20
41, 64	2,2′,3,4′; 2,3,4′,6	4.0	1.0	3.4	0.8
44	2,2′,3,5′	2.4	1.3	2.7	0.8
49	2,2′,4,5′	2.8	0.7	2.0	<LOD
52	2,2′,5,5′	6.0	4.1	7.4	2.0
56, 60	2,3,3′,4′; 2,3,4,4′	1.3	1.6	6.2	<LOD
66	2,3′,4,4′	5.4	5.6	12.7	6.4
70	2,3′,4′,5	4.2	3.5	5.2	2.1
74	2,4,4′,5	2.4	3.2	6.3	2.9
82	2,2′,3,3′,4	<LOD	<LOD	0.8	<LOD
84	2,2′,3,3′,6	1.1	1.0	1.1	<LOD
85	2,2′,3,4,4′	1.7	2.4	3.1	<LOD
87, 115	2,2′,3,4,5′; 2,3,4,4′,6	3.2	1.3	2.8	3.0
90, 101	2,2′,3,4′,5; 2,2′,4,5,5′	7.9	3.8	4.6	2.6
91	2,2′,3,4′,6	0.9	<LOD	<LOD	<LOD
92	2,2′,3,5,5′	2.1	2.2	1.6	2.5
95	2,2′,3,5′,6	5.8	4.6	4.5	3.4
97	2,2′,3′,4,5	2.4	1.1	1.1	<LOD
99	2,2′,4,4′,5	4.8	5.0	<LOD	6.2

105	2,3,3′,4,4′	3.1	3.0	11.6	2.9
110	2,3,3′,4′,6	10.4	6.8	<LOD	3.0
118, 123	2,3′,4,4′,5; 2′,3,4,4′,5	6.5	7.4	9.6	8.9
132	2,2′,3,3′,4,6	1.8	<LOD	1.1	<LOD
135	2,2′,3,3′,5,6′	1.3	1.8	<LOD	<LOD
136	2,2′,3,3′,6,6′	<LOD	0.6	<LOD	<LOD
138	2,2′,3,4,4′,5′	5.5	14.1	5.0	18.7
146	2,2′,3,4′,5,5′	2.1	2.6	0.1	3.8
149	2,2′,3,4′,5′,6	3.6	5.6	1.3	5.4
151	2,2′,3,5,5′,6	1.7	<LOD	<LOD	2.9
153	2,2′,4,4′,5,5′	5.6	15.1	5.6	20.7
176	2,2′,3,3′,4,6,6′	<LOD	0.6	<LOD	0.9

LOD = level of detection.

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